REMARKS

The Final Office Action of March 28, 2001 presents the Examination of claims 52-73 and 76-79. A Request for Continued Examination was filed on September 28, 2001. No amendments were made at that time and the claims remained as examined on March 28, 2001.

An interview with the Examiner and her supervisor was held on November 16, 2001. The cooperation of the Examiner in advancing the prosecution of the present application is greatly appreciated. This paper reflects the substance of the interview.

Objection to the specification

The Examiner objects to the specification, indicating that the plasmids referred to in the figures must be identified by SEQ ID NO. This is not correct. The plasmids are descriptions of recombinant nucleic acid constructs; no nucleotide or amino acid sequence is presented in the figures. Accordingly, it is not necessary to identify the plasmids by SEQ ID NO.

Figure 10A-C presents a nucleotide sequence. This sequence is presented in the Sequence Listing of 6 pages filed with the

original application papers. The description of Figures 10A-C of the specification is amended to recite SEQ ID NO: 1.

Rejections under 35 U.S.C. § 112, second paragraph

Claim 57 stands rejected under 35 U.S.C. § 112, second paragraph as being a Markush claim in improper form. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

Claim 57 is not a "Markush" style claim. All of the elements recited in claim 57 are included in one claimed embodiment, not as alternative embodiments. Thus, claim 57 is correct as written.

Claim 63 stands rejected under 35 U.S.C. § 112, second paragraph, as improperly dependent upon claim 62. The Examiner correctly points out that claim 63 recites "adeno-associated" virus. This was an error in writing the claim. Claim 63 is amended to correctly recite "adenovirus" and so is now correctly dependent upon claim 62, thus obviating this rejection.

Claim 69 is rejected under 35 U.S.C. § 112, second paragraph, for failure to recite a final step relating back to the preamble. Claim 69 is amended to recite such a final step, thus obviating this rejection.

Claim 70 is rejected under 35 U.S.C. § 112, second paragraph, for lack of antecedent basis for the term "subject" in the base claim 69. Claim 69 is amended to provide the required antecedent basis, thus obviating this rejection.

Rejection under 35 U.S.C. § 112, first paragraph

Claims 69-71 and 76-77 stand rejected under 35 U.S.C. § 112, first paragraph, for alleged lack of enablement of the claimed invention by the specification. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

The Examiner indicates that the specification does not enable compositions "expressed in any whole organism via any route of administration as broadly claimed." Applicants submit that the Examiner has overlooked some limitations in the claims in making this rejection.

First, as to claims 69-71, the claims are directed to methods for delivery of a gene, not to compositions. Second, as to all of the rejected claims, the claimed method for gene delivery is for delivery to cardiac tissue, not to "any whole organism". (With respect to claims 76-77, this aspect comes from claim 52, from which these claims ultimately depend.)

The specification provides working examples showing that a vector according to the present invention can be administered to a whole animal, with the result that expression of a marker gene only in cardiac tissue is obtained. Applicants submit that one of ordinary skill in the art can follow the teachings of the specification, in particular making a construct for expressing the desired nucleic acid that drives that expression using the control elements as described in the specification, administer the construct in the way described in the specification and obtain specific expression of a desired gene specifically in the cardiac tissue of a whole animal. That is all that is necessary for the specification to be enabling of the invention.

In the interview, the Examiner agreed that, at least the claims to a method for gene delivery to cardiac tissue were considered enabled on the basis of a showing of exactly that in the working examples.

For all of the above reasons, Applicants submit that the instant rejection should be withdrawn.

Rejections over prior art

Claims 52-68, 72-73 and 76-77 stand rejected under 35 U.S.C. § 103(a) over Franz et al., Arnold et al., Knowleton et al., Shubeita et al, Navankasattusas et al, Thornburn et al.,

Goswami et al and Ricigliano et al, in view of Zaia et al and Posakoff et al. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

In the interview of November 16, 2001, Applicants made the point that none of the cited references, alone or in combination, establish *prima facie* obviousness of the invention, or alternatively that the specification includes data that constitute and unexpected result sufficient to rebut an assertion of *prima facie* obviousness.

The focus of the discussion was first upon the Franz reference. In the Franz reference, a construct of the 2.1 kb upstream region of the MLC gene was linked to a luciferase gene and transferred into transgenic mice through the germ line by oocyte injection. Thus, the gene was present in every cell of the body of the transgenic mouse. Expression of the construct was assessed and cardiac specific expression was found.

On the other hand, the present claims recite that portions of an MLC upstream region that are sufficient for cardiac-specific expression of a desired nucleic acid after administration to somatic cells are linked to that nucleic acid in a viral, preferably an adenoviral or adeno-associated virus, vector. The state of the art at the time the invention was made was such that successful cardiac-specific expression obtained by

germ line transmission in no way established that the same sort of construct would provide successful cardiac-specific expression of the same gene when administered to somatic cells.

One apparent cause of this unpredictability lies in the use of adenovirus type vectors. As explained during the interview, adenoviruses (and many other mammalian viruses such as lentiviruses) include as part of their genome an inverted terminal repeat sequence (ITR or LTR) and various enhancer elements. It appears that often these terminal repeats and enhancers interfere with specificity elements of the inserted gene constructs, leading to unexpected changes in the tissue specificity of expression of exogenous nucleic acids incorporated into constructs in the vector. As evidence of this, Applicants provide Exhibit 1, copies of two abstracts of papers that discuss effects of the adenovirus ITR on tissue specificity. The abstract by Shin et al. shows that an adenovirus ITR has a negative effect on overall expression of a transgene driven by the myosin light chain promoter 1. The abstract by Rubinchik shows that the E1A portion of the adenovirus genome appears to decrease selectivity of expression of transgene promoters.

Another aspect of unpredictability in obtaining cardiac tissue-specific expression is that the particular combination of

elements of the upstream region used by Franz et al. in their germ line experiment that confer cardiac-specific expression were not identified. This is evident from the Navankasattusas paper cited by the Examiner. In Figure 9 of that paper, constructs using two or three of the elements from the upstream region of an MLC promoter, in various combinations, were used as substrates in gel shift assays to see what proteins might be bound from extracts made from skeletal muscle and from cardiac muscle cells. The authors found that in fact identical proteins were bound by the constructs in both cell types. The constructs were transfected into cardiac cells and expression of a marker gene was observed. However, there was no corresponding experiment performed in skeletal muscle cells. Thus, the reader is left with the impression that the combinations tried by Navankasattusas are insufficient to establish cardiac-specific tissue specificity of expression. Accordingly, and relevant to the present claims reciting particular nucleic acid segments, one of ordinary skill in the art would not know what combinations of small nucleic acid elements confer cardiac tissue specificity to gene expression at the time the present invention was made.

As further evidence of unpredictability, Applicants attach Exhibit 2, a paper by Buttrick et al.. This paper shows that an

upstream sequence shown to confer thyroid hormone responsiveness to gene expression in cardiomyocytes in cell culture does not do so when transformed into cardiac tissue *in vivo*.

The most important evidence of unpredictability, which might alternatively be considered an unexpected experimental result, is provided by the present specification. The myosin heavy chain (MHC) promoter and the myosin light chain (MLC) promoter, at the time the present application was filed, were both considered, based upon germ line transgenic animal experiments and other experiments, to confer cardiac tissue specificity upon gene expression. However, when both of these promoters were incorporated into a viral vector according to the present invention, only the MLC promoter was found to confer cardiac tissue-specific expression of a marker gene. (*See*, Examples 1 (vector constructions) and 11 and the data in Figures 8A and 8B.) Significant expression of the marker is seen in lung and liver when the MHC promoter is used. However, only cardiac tissue expresses the marker when the MLC promoter is used.

For all of the above reasons, Applicants submit that the invention as recited in claims 52-68, 72-73 and 76-77 is not obvious in view of the cited references. Accordingly, the instant rejection should be withdrawn.

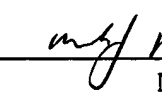
Applicants submit that the present application well-describes and claims patentable subject matter. The favorable action of allowance of the pending claims is respectfully requested.

If there are any minor matters precluding allowance of the application which may be resolved by a telephone discussion, the Examiner is asked to contact Mark J. Nuell, Ph.D. (Reg. No. 36,623) at (703) 205-8000.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By 

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Attachments: **Mark-up version showing changes**
Declaration
Exhibits 1 and 2

Mark-up Version Showing Changes Made

In the Specification

The second paragraph beginning on page 15, line 7 has been amended as follows:

--Figs. 10A-C show the nucleic acid sequence of a 2216 base pair-long promoter of the MLC-2v gene of rats (SEQ.ID. NO:1), lying upstream of the transcription starting point (+1). The nucleic acids of positions 1-156 encode for the packaging sequence ψ of adenovirus Ad5 (positions 300-456). The cloning sequence for the restriction endonuclease BamHI is located at position 158-163 and for KpnI at positions 189-194. At position 189-2405 is located the 2216 base pair-long promoter of the MLC-2v gene. The CSS-like sequence is located at position 682-724, the HF element at position 2207-2219, the MLE1 element at position 2229-2241, the HF2 element at position 2271-2289, the E box element at position 2328-2333, the HF 1a element at position 2340-2348, the HF 1b element at position 2349-2361 and the transcription start (+1) at position 2406. The luciferase encoding sequence starts at position 2461. At position 1660-2406 lies the 746 base pair-long regulatory sequence of the plasmid pAd-mlcLuc (see Example 1).

63. (Amended) The recombinant virus vector according to claim 62, wherein said replication deficient [adeno-associated] adenovirus [virus] vector consists of two inverted terminal repetition sequences [(ITR)].

69. (Amended) A method for delivery of a desired gene to cardiac muscle cells of a subject, which method comprises administering a recombinant virus vector according to claim 52, optionally complexed with liposomes, to cardiac tissue of a subject, thereby delivering the desired gene to the cardiac muscle cells of the subject.